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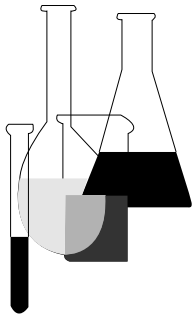
Health Effects Test Guidelines

OPPTS 870.8380 Inhalation and Dermal Pharmacokinetics of Commercial Hexane



"Public Draft"

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

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OPPTS 870.8380 Inhalation and dermal pharmacokinetics of commercial hexane.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is OPPT 40 CFR 795.232 Inhalation and Dermal Pharmacokinetics of Commercial Hexane.

(b) **Purpose**. The purpose of these studies is to determine the bioavailability of the test substances after dermal and inhalation administration; to compare the pharmacokinetics and metabolism of the test substances after intravenous, dermal, and inhalation administration; and to examine the effects of repeated doses on the pharmacokinetics and metabolism of the test substances.

(c) **Definitions**. The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definitions also apply to this test guideline.

Bioavailability refers to the relative amount of administered test substance which reaches the systemic circulation and the rate at which this process occurs.

High dose should not exceed the lower explosive limit (LEL) and should induce minimal toxicity.

Low dose should correspond to one-tenth of the high dose.

Metabolism means the sum of the enzymatic and nonenzymatic processes by which a particular substance is handled in the body.

Pharmacokinetics means the study of the rates of absorption, tissue distribution, biotransformation, and excretion.

Test substance refers to the unlabeled and both radiolabeled mixtures (^{14}C-*n*-hexane and ^{14}C-methylcyclopentane (MCP)) of commercial hexane used in the testing.

(d) **Test procedures**—(1) **Animal selection**—(i) **Species**. The rat should be used for pharmacokinetics testing because it has been used extensively for metabolic and toxicological studies.

(ii) **Test animals**. Adult male and female rats should be used for testing. The rats should be 7 to 9 weeks old and their weight range should be comparable from group to group. The animals should be purchased from a reputable dealer and should be permanently identified upon arrival.

The animals should be selected at random for the testing groups, and any animal showing signs of ill health should not be used.

(iii) **Animal care**. (A) Animal care and housing should be in accordance with DHHS/PHS NIH Publication No. 86–23 (1985), Guidelines for the Care and Use of Laboratory Animals.

(B) The animals should be housed in environmentally controlled rooms with at least 10 air changes per hour. The rooms should be maintained at a temperature of 18 to 26 °C and humidity of 40 to 70 percent with a 12–h light/dark cycle per day. The animal subjects should be kept in a quarantine facility for at least 7 days prior to use, and should be acclimated to the experimental environment for a minimum of 48 h prior to treatment.

(C) During the acclimatization period, the rats should be housed in suitable cages. All animals should be provided with certified feed and tap water ad libitum.

(2) **Administration of test substances**—(i) **Test substances**. The study will require the use of both radiolabeled and unlabeled test substances. All unlabeled commercial hexane should be from the same lot number. Two kinds of radiolabeled test substances will be tested. ^{14}C-*n*-hexane should be the only radiolabeled component of one, and ^{14}C-MCP should be the only radiolabeled component of the other test substance. The use of both radiolabeled test substances is required for all pharmacokinetics and metabolism studies described in this rule, except for the bioavailability measurements required in paragraph (d)(4)(i)(A) of this guideline. The bioavailability measurements need only be conducted with the test substance containing ^{14}C-*n*-hexane or an unlabeled test substance may be used if it can be demonstrated that the analytical sensitivity of the method used with the unlabeled test substance is equal to or greater than the sensitivity which could be obtained with the radiolabeled test substance. If an unlabeled test substance is used for bioavailability measurements, these measurements should be extended to include relevant metabolites of *n*-hexane. These test substances should contain at least 40 liquid volume percent but no more than 55 liquid volume percent *n*-hexane and no less than 10 liquid volume percent methylcyclopentane (MCP) and otherwise conform to the specifications prescribed in the American Society for Testing and Materials Designation D 1836–83 (ASTM D 1836), Standard Specification for Commercial Hexanes.Copies of this material may be obtained from the American Society for Testing and Materials (ASTM), 1916 Race Street, Philadelphia, PA 19103.

(ii) **Dosage and treatment**—(A) **Intravenous**. An appropriate dose of the test substance should be administered intravenously. The intravenous data obtained in this portion of the study should be suitable for

the determination of absorption, distribution, and excretion parameters of the test substance. Factors that should be considered in the selection of the intravenous doses are: The acute toxicity of the test substance, the availability of a suitable vehicle (if saline is unsuitable) and the solubility of the test substance in the vehicle.

(B) **Inhalation**. Two concentrations of each test substance should be used in this portion of the study, a low concentration and a high concentration. The high concentration should induce minimal toxicity, but should not exceed the LEL. The low concentration should correspond to one-tenth of the high concentration. Inhalation treatment should be conducted using a nose-cone or head-only apparatus to reduce ingestion of the test substance through grooming or dermal absorption.

(C) **Dermal**. Dermal absorption studies should be conducted as described under paragraph (f)(1) of this guideline or by some other suitable method, care being taken because of the significant volatility of the test substances. The high and low doses should be tested in rats.

(iii) **Dosing and sampling schedule**. Each experimental group should contain at least four animals of each sex. After administration of the test substance, each rat should be placed in an individual metabolic unit for collection of urine, feces, and expired air. For the dermal studies, excreta from the rats should also be collected during the exposure periods. At the end of each collection period, the metabolic units should be cleaned to recover any excreta that might adhere to the units. All studies, except the repeated dose studies, should be terminated at 7 days, or after at least 90 percent of the administered radioactivity has been recovered in the excreta, whichever occurs first. All studies described below should be conducted separately with each radiolabeled test substance.

(A) **Intravenous study**. Group A should be given a single intravenous dose of the radiolabeled test substance to result in a level of commercial hexane in the blood that approximates the level from the other routes of exposure so that the data can be used to determine absorption and excretion parameters.

(B) **Inhalation studies**. A single 6–h exposure period should be used for each group.

(*1*) Group B should be exposed to a mixture of the radiolabeled test substance in air at the low concentration.

(*2*) Group C should be exposed to a mixture of the radiolabeled test substance in air at the high concentration.

(C) **Dermal studies**. The test substance should be applied and kept on the skin for a minimum of 6 h. The covering apparatus components should be assayed to recover residual radioactivity. At the termination of

the studies, each animal should be sacrificed and the exposed skin area removed. An appropriate section of the skin should be solubilized and assayed for radioactivity to ascertain whether the skin acts as a reservoir for the test substance.

(*1*) Group D should be given one dermal, low dose of the radiolabeled test substance.

(*2*) Group E should be given one dermal, high dose of the radiolabeled test substance.

(D) **Repeated dosing study**. Group F should receive a series of single daily 6–h inhalation exposures to unlabeled test substance at the low dose over a period of at least 7 days. A single 6–h inhalation exposure to the radiolabeled test substance at the low dose should be administered 24 h after the last unlabeled exposure. Following administration of the radiolabeled substance, the rats should be placed in individual metabolic units and excreta collected. The study should be terminated 7 days after the last exposure, or after at least 90 percent of the radioactivity has been recovered in the excreta, whichever occurs first.

(3) **Types of studies**—(i) **Pharmacokinetics studies**. Groups A through F should be used to determine the kinetics of absorption of the test substance. In animal subjects administered the test substance intravenously (i.e. Group A), the concentration of test substance in blood and excreta should be measured following administration. In animal subjects administered the test substance by the inhalation and dermal routes (i.e. Groups B through F), the concentration of test substance in blood should be measured at selected time intervals during and following the exposure period. In animal subjects administered the test substance by the inhalation route (i.e. Groups B, C, and F) the concentration of test substance in excreta should be measured following exposure. In animal subjects administered the test substance by the dermal route (i.e. Groups D and E) the concentration of test substance in excreta should be measured during and following exposure. These measurements allow calculation of uptake, half lives, and clearance. In addition, in the groups administered the test substance by inhalation (i.e. Groups B, C, and F), the concentration of test substance in the exposure chamber air should be measured at selected time intervals during the exposure period.

(ii) **Metabolism studies**. Groups A through F should be used to determine the metabolism of the test substance. Excreta (urine, feces, and expired air) should be collected for identification and measurement of the quantities of test substance and metabolites.

(4) **Measurements**—(i) **Pharmacokinetics**. At least four animals from each group should be used for these purposes.

(A) **Bioavailability**. The levels of test substance and relevant metabolites, as appropriate, should be determined in whole blood, blood plasma or blood serum at appropriate intervals after initiation of intravenous, dermal, and inhalation exposure. The sampling intervals should be compatible with the exposure route under study. The determinations need only be done on animals administered the test substance containing ^{14}C-*n*-hexane or, if the analytical sensitivity is equal or greater, unlabeled test substance may be used.

(B) **Extent of absorption**. The total quantities of radioactivity should be determined for excreta collected daily for 7 days, or until at least 90 percent of theradioactivity has been recovered in the excreta, whichever occurs first.

(C) **Excretion**. The quantities of radioactivity eliminated in the urine, feces, and expired air should be determined separately at time intervals that provide accurate measurement of clearance and excretory rates. The collection of carbon dioxide may be discontinued when less than one percent of the dose is found to be exhaled as radioactive carbon dioxide in 24 h.

(D) **Tissue distribution**. At the termination of each study, the quantities of radioactivity should be determined in blood and in various tissues, including bone, brain, fat, gastrointestinal tract, gonads, heart, kidney, liver, lungs, muscle, skin, spleen, thymus, and residual carcass of each animal.

(E) **Change in pharmacokinetics**. Results of pharmacokinetics measurements (i.e. biotransformation, extent of absorption, tissue distribution, and excretion) obtained in rats receiving the single inhalation exposure to the low dose of the test substance (Group B) should be compared to the corresponding results obtained in rats receiving repeated inhalation exposures to the low dose of the test substance (Group F).

(ii) **Metabolism**. At least four animals from each group should be used for these purposes.

(A) **Biotransformation**. Appropriate qualitative and quantitative methods should be used to assay urine, feces, and expired air collected from rats. Efforts should be made to identify any metabolite which comprises 5 percent or more of the dose administered.

(B) **Changes in biotransformation**. Appropriate qualitative and quantitative assay methods should be used to compare the composition of radioactive compounds in excreta from rats receiving a single inhalation exposure (Groups B and C) with that from rats receiving repeated inhalation exposures (Group F).

(e) **Data and reporting**. The final test report should include the following:

(1) **Presentation of results**. Numerical data should be presented in tabular form. Pharmacokinetics data should also be presented in graphical form. Qualitative observations should also be reported.

(2) **Evaluation of results**. All data should be evaluated by appropriate statistical methods.

(3) **Reporting results**. In addition to the reporting requirements as specified in 40 CFR part 792, the following information should be reported.

(i) Strain of laboratory animals used.

(ii) Chemical characterization of the test substances, including:

(A) For the radiolabeled test substances, information on the sites and degree of radiolabeling, including type of label, specific activity, chemical purity prior to mixing with the unlabeled hexane mixture, and radiochemical purity.

(B) For the unlabeled test substance, information on lot number and the percentage of MCP and *n*-hexane.

(C) Results of chromatography.

(iii) A full description of the sensitivity, precision, and accuracy of all procedures used to obtain the data.

(iv) Percent and rate of absorption of the test substance after inhalation and dermal exposures.

(v) Quantity and percent recovery of radioactivity in feces, urine, expired air, and blood. For dermal studies, include recovery data for skin and residual radioactivity in the covering apparatus.

(vi) Tissue distribution reported as quantity of radioactivity in blood, in various tissues including bone, brain, fat, gastrointestinal tract, gonads, heart, kidney, liver, lung, muscle, skin, spleen, thymus, and in residual carcass.

(vii) Biotransformation pathways, to the extent possible, and quantities of the test substances and metabolites in excreta collected after administering single high and low doses.

(viii) Biotransformation pathways, to the extent possible, and quantities of test substances and metabolites in excreta collected after administering repeated low doses.

(ix) Pharmacokinetics models to the extent they can be developed from the experimental data.

(f) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Susten, A.S. et al. In vivo percutaneous absorption studies of volatile solvents in hairless mice. I. Description of a skin depot. *Journal of Applied Toxicology* 6:43–46 (1986).

(2) [Reserved]